Agonistic Interactions Between Colonies of the Formosan Subterranean Termite (Isoptera: Rhinotermitidae) in New Orleans, Louisiana

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ABSTRACT Our objective was to examine the pattern of agonistic behavior observed among 11 colonies of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, collected from two locations in New Orleans, LA. In 24-h petri dish tests, 13 of the 40 colony pairs displayed agonism in at least one replicate. In 14-d tests conducted in sand-filled foraging arenas, seven of the nine colony pairs tested showed agonism, including three that had showed agonism in the 24-h test and four that had not shown any agonism in the 24-h test. In tests in which individual behavior was observed for 1 h, there was variation in the prevalence of aggressive behavior shown by individual workers. This study shows that interactions between colonies of *C. formosanus* from the same geographic area are complex and that these interactions could influence strategies for termite control.

KEY WORDS *Coptotermes formosanus*, Rhinotermitidae, agonistic behavior, kin recognition, aggression.

THE FORMOSAN SUBTERRANEAN TERMITE, *Coptotermes formosanus* Shiraki, is an exotic pest species, originating in East Asia, that has successfully invaded numerous places around the world, including Japan, HI, South Africa, and the continental United States (Su and Tamashiro 1987). Exotic social insects often have particular attributes that contribute to their ability to successfully invade new habitats. For example, a number of exotic ant species are characterized by their lack of intraspecific aggression, and their ability to exchange workers and queens between nests. The Argentine ant, *Linepithema humilis* (Mayr), is a worldwide pest that has been extremely successful colonizing new habitats and outcompeting native ant species. The success of Argentine ants appears to be primarily due to the extensive movement of workers and queens between nests, which results in a competitive advantage over native ant species (Chen and Nonacs 2000).

Formosan subterranean termites have also been extremely successful becoming established in new habitats. In some cases, there has been a lack of intraspecific aggression in introduced populations of *C. formosanus* (Delaplane 1991, Su and Haverty 1991). This lack of aggression between colonies could potentially contribute to the success of *C. formosanus* as an invasive pest species by enabling *C. formosanus* to establish large populations, with extensive networks of tunnels to efficiently use food sources. This study examined the prevalence of agonistic interactions among *C. formosanus* colonies in New Orleans, LA.

The occurrence of agonism in subterranean termites varies among species, colonies, nests, castes, individuals, and seasons (Thorne and Haverty 1991, Shelton and Grace 1996). Intraspecific agonism between colonies of subterranean termites is extremely variable, ranging from no observed agonism to fierce combat. The occurrence of intraspecific agonism among *Reticulitermes lucifugus* (Rossi) colonies varied by season in which intercolonial agonism was high in the winter and decreased in the summer, but no agonism was observed between colonies of *R. santonensis* Feytaud (Clément 1986). In some cases, studies have shown a lack of intraspecific agonism among different colonies of *R. flavipes* (Kollar) collected at different sites in the same geographic area (Grace 1996, Bulmer and Traniello 2002). Agonism among *C. formosanus* colonies collected from the campus of the University of Hawaii was observed for some colony pairs, but not others, while none of the colonies collected from Hallendale, FL, showed agonism towards each other (Su and Haverty 1991). When six colonies from Florida were paired with six colonies from Hawaii, agonistic responses were variable, with no agonism observed when three of the Florida colonies were paired with any of the Hawaii colonies (Su and Haverty 1991).

Fierce combat was observed between members of different colonies of *Heterotermes aureus* (Snyder) in laboratory tests, and large numbers of dead termites

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locked in combat were found in corrugated-paper rolls located on the boundary between two of the colonies (Jones 1993). Intercolonial agonism was also observed among colonies of *Reticulitermes* spp. from northern California. Mixing workers with different cuticular hydrocarbon phenotypes resulted in immediate aggressive behavior in 48.8% and 61.5% of replicates, while mixing workers from different colonies with the same cuticular hydrocarbon phenotype usually did not result in immediate aggression but resulted in high mortality after 24 h in 68.6% of replicates (Haverty et al. 1999).

The variability in intercolonial agonism would be expected to influence the foraging behavior and the size of foraging ranges of neighboring colonies in the field. In the absence of intercolonial agonism, there is the possibility of colony fusion. Colony fusion may have occurred between two colonies of *C. formosanus* in Florida (Su and Scheffrahn 1988). Colony fusion could provide a mechanism for *C. formosanus* to rapidly increase colony size and expand the foraging range of the new colony. It is possible that the extremely large colony sizes reported in the field for *C. formosanus* could sometimes be the result of the fusion of neighboring colonies that did not have any agonistic behavior towards each other. Alternatively, competition between agonistic colonies could result in the maintenance of smaller colonies and retention of smaller foraging ranges. For example, agonistic interactions between field colonies of *H. aureus* appeared to affect colony foraging territories, where intercolony aggression resulted in gaps between territories (Jones 1993). Interactions between colonies of *C. formosanus* could have an effect on strategies for termite control by influencing the foraging behavior of termites at bait stations.

In this study, we examined the patterns of agonistic behavior between pairs of 11 colonies of *C. formosanus* collected from two locations in New Orleans, LA. Also, experiments were conducted to determine how five colonies from the same area interacted with each other in sand-filled test chambers, and how agonistic behavior affected their foraging ranges and survival. In addition, experiments were performed, using marked individuals, in which workers of two colonies were paired to determine if there were differences in the agonistic behavior displayed by individuals toward workers of other colonies.

Materials and Methods

Twenty-Four-h Petri Dish Tests. Tests were conducted to determine which colony pairs showed agonistic behavior towards each other that resulted in mortality. These tests were not designed to detect nonfatal agonistic encounters. For these tests, termites were used within 2 wk of being collected. There were 11 colonies of *C. formosanus* collected from two different locations in New Orleans, LA. Seven colonies (C1, C2, C5, C6, C10, C11, C12) were collected from the campus of the Southern Regional Research Center, Agricultural Research Service, USDA, and within a 1,000 m^2 area on the periphery of the campus. Four colonies (C7, C9, C14, C15) were collected from the University of New Orleans campus. Voucher specimens of soldiers and workers of each termite colony are stored in 70% alcohol at the Southern Regional Research Center, New Orleans, LA.

Collections from different traps were considered separate colonies based on the use of the mark-release-recapture technique using the dye marker Nile Blue A (0.05% wt:wt) to determine those traps, which were part of a single, interconnected tunneling system (Su et al. 1993). Termites collected from traps that exchanged foraging workers were considered part of the same colony.

For each replicate, 10 workers from each of two colonies were placed in a 5.5-cm diameter glass petri dish, with a 5.5-cm diameter moist filter paper on the bottom and kept in an unlit environmental chamber at 28°C, 97% RH overnight. There were 40 colony pairs, with at least eight replicates for each pair, except in the cases of C1:C2, C2:C6, C6:C10 pairings, for which there were only four replicates each. There were four control replicates for each colony. For controls, 20 workers from the same colony were placed in a petri dish for 24 h. After 24 h, the number of termites surviving in each replicate was determined. There were no controls in which survival was < 17. For colony pairs, the replicate was scored as agonistic if the total number of termites surviving was < 15. In addition, in replicates scored as agonistic, dead termites showed signs of injury due to combat.

Fourteen-d Colony Interaction Test. This experiment was conducted using five colonies (C1, C5, C6, C11, C12) collected within a 1,000 m^2 area on the periphery of the campus of the Southern Regional Research Center in New Orleans, LA. The experiment was conducted in the laboratory at ambient conditions, using plastic ant farms (21.0 cm length by 1.0 cm width by 13.5 cm height) (Uncle Milton Industries, Corsica, CA) as test chambers. Each test chamber (ant farm) contained 100 g of sand (Standard Sand and Silica Company, Davenport, FL), moistened with 20 ml of distilled water, and four pieces of southern yellow pine, *Pinus taeda* L., (10 cm length by 0.1 cm width by 3.5 cm height). Test chambers were placed on a shelf in the laboratory that was completely enclosed in black plastic to block out direct light.

Each replicate was comprised of three connected test chambers. A chamber containing a group of termites from one colony was connected to an unoccupied chamber, and a chamber containing a group of termites from another colony was connected to the opposite side of the same unoccupied chamber. There were 180 workers and 20 soldiers of each colony in each replicate. There were four replicates of each colony pair. For controls, there were eight replicates of each colony where a test chamber containing 180 workers and 20 soldiers from a single colony was connected to one side of an unoccupied center chamber and another unoccupied test chamber was connected to the opposite side of the center chamber. For each colony pair, one group of workers in each replicate was fed filter paper dyed with 0.05% Nile Blue A dye (wt:wt) for 1 wk before starting the experiment so that workers from the two colonies could be distinguished. In two of the replicates, workers from one colony were dyed blue, and in the other two replicates, workers from the other colony were dyed blue.

Termites were placed in the top of test chambers, and allowed to acclimate and build tunnels in the sand for 1 d before chambers were connected. Each test chamber had a portal on each side, covered with a plastic cap. Chambers were connected by removing the cap and attaching the ends of a 4-cm length piece of tygon tubing (0.8 cm diameter) to the portals of two chambers. After 14 d, the number of surviving workers from each colony in each chamber was counted. Soldiers were counted separately, without determining colony origin. If 10 or more workers of a colony were present in a chamber, the chamber was considered to be occupied by that colony.

Differences in worker survival of each colony within a colony pair were compared using a *t*-test for matched pairs ($P \leq 0.05$). Also, proportional worker survival data were transformed by the arcsine of the square root and the following three statistical tests were conducted, using General Linear Models, with mean separated using the Tukey honestly significant difference test (SPSS 1996). In the first GLM test, differences in the worker survival of each colony in the controls were compared. In the second GLM test, differences in worker survival were compared for controls and for each colony pair. In the third GLM test, colony pairs were divided into three groups based on observations of agonistic behavior in both the 24-h test and the 14-d test. Differences in worker survival in the 14-d test were compared for the following four groups (1) controls; (2) colony pairs that did not show agonism in either test; (3) colony pairs that did not show agonism in the 24-h petri dish test but did show agonism in the 14-d colony interaction test; and (4) colony pairs that exhibited agonism in both tests. Soldiers were not included in the statistical analysis because the colony origin of soldiers could not be determined in most of the replicates.

Intercolonial Interactions between Individually Marked Termites. For each replicate, six workers from each of two colonies were placed in a 5.5-cm diameter glass petri dish, with a 5.5 cm diameter moist filter paper on the bottom. The filter paper was positioned so that the termites were not able to go underneath it. Each individual termite had a distinctive mark so that the aggressive behavior of individuals could be monitored. Termites in each replicate were observed for 1 h. The number of individuals in each replicate that attacked individuals from the other colony by biting them was recorded. These attacks could be distinguished from other nonaggressive encounters. Aggressive encounters were only recorded when individuals were actually biting other individuals. There were nine different colony pairs, with 10 replicates for each colony pair.

Individuals of one colony were coded using white, blue, pink, and canary yellow liquid paper (The Gillette Company, Boston, MA), and individuals of the other colony were coded by using the following Van Gogh water colors (Royal Talens, Apeldoorn, Holland): Ivory black, permanent red light, viridian, phthalo blue, and quinacridone rose. For each colony pair, in half the replicates, one colony was marked with liquid paper, and in the other half of the replicates, that colony was marked with the watercolors.

To mark termites, a glass petri dish was placed on top of a container with crushed ice. An individual termite was placed in the petri dish for ≈10 s. When the chilled termite was lying on its back and unable to get up, it was allowed to grasp onto a wooden stick. A dot of paint was gently applied to the abdomen of the termite, using the end of an insect pin. After being marked, the termite was allowed to walk down the stick into a petri dish. Termites were not marked when they were totally immobilized because the procedure was less likely to damage the termite if the termite could grasp the stick while the dot of paint was being applied.

When a dot of liquid paper was placed on an individual termite, other workers would attempt to groom it off, but they could not remove it. In fact, individual soldiers marked with a dot of liquid paper were returned to a container with thousands of unmarked nestmates and still retained this mark after 2 mo. When a dot of watercolor was placed on a worker, other workers would ingest small quantities of paint when grooming it off. After several hours, the abdomens of all workers in the petri dish would be colored because of ingesting the paint. There were no differences in mortality between workers that were marked by either method and unmarked workers (M.L.C., unpublished data).

For these tests, six workers from one colony were marked with liquid paper and were distinguished from each other by differences in color (except that two workers in each replicate were distinguished from another worker with the same color by differences in the size of the mark). Five workers from the other colony were each marked with a different watercolor, and the sixth worker was unmarked.

Results

Twenty-Four-h Petri Dish Tests. Of 40 colony pairs, 13 (33%) showed agonism in at least one replicate (Table 1). Of the 13 colony pairs that displayed agonism, five of these pairs were agonistic in 100% of replicates, eight involved SRRC:UNO colony pairs, four involved SRRC:SRRC colony pairs, and one involved a UNO:UNO colony pair. All SRRC:SRRC colony pairs that showed agonism involved C11. Only a single replicate of six UNO:UNO colony pairs displayed agonism. Of the colony pairs that resulted in 60% or more of replicates showing agonism, all these pairs involved at least one of the following colonies: C1, C9, or C11 (Table 1).

Fourteen-d Colony Interaction Test. There were no significant colony differences in worker survival in controls ($F = 0.525$; df = 4, 33; $P = 0.72$) (Table 2).

Table 1. Percent replicates of *C. formosanus* colonies displaying agonism in 24-h petri dish tests

Colony Location	C1 S	C2 S	C5 S	C6 S	C7 U	C9 U	C10 S	C11 S	C12 S	C14 U	C15 U
C1	0	0	0	0	0	62.5	0	100	0	100	37.5
C2		0	0	0	–	–	0	100	–	–	–
C5			0	0	0	100	–	18	0	0	–
C6				0	0	100	0	42	0	0	–
C7					0	0	0	12.5	–	0	12.5
C9						0	–	75	–	0	0
C10							0	0	25	0	0
C11								0	0	0	0
C12									0	–	–
C14										0	0
C15											0

S, the campus of the Southern Regional Research Center and the area on the periphery of the campus; U, the campus of the University of New Orleans.

There was only one colony pair (C5/C11) in which there were significant differences in the survival of workers from the two colonies (*t*-test for matched pairs; $P \leq 0.05$). Of the nine colony pairs tested, seven pairs showed agonism in these tests, including three pairs that had showed agonism in the 24-h test and four pairs that had not exhibited any agonism in the 24-h test. There were significant differences in the survival of workers from the controls and from the different colony pairs ($F = 19.935$; df = 9, 100; $P = 0.0001$). There were four colony pairs in which workers of both colonies had very high levels of mortality. Two of these pairs had shown agonism in both tests, while the other two pairs only showed agonism in the 14-d test. In fact, the colony pair that had the lowest worker survival did not show any agonism in the 24-h petri dish test.

There were significant differences in worker survival based on the level of agonism observed between colony pairs in 24-h and 14-d tests ($F = 23.13$; df = 3, 106; $P = 0.0001$). Worker survival was significantly lower for colony pairs that showed agonism in the 14-d test, compared with colony pairs that did not show any agonism (Fig. 1). However, there were no differences between colony pairs that showed agonism in only the 14-d test, compared with colony pairs that showed agonism in both tests. Also, there were no differences between the controls and colony pairs that did not display any agonism (Fig. 1).

The movement of termites within test chambers was also affected by the level of agonism observed between colony pairs in the 24-h and 14-d tests. In controls, termites generally explored all three test chambers, but the majority of termites settled into one chamber. For colony pairs that did not show any agonism in either the 24-h or 14-d tests, one colony moved into the other colony's chamber in 68.8% of replicates. In almost all of these replicates, termites from the two colonies intermingled, and termites from one colony traveled within the tunnels constructed by workers from the other colony. In some replicates, termites from one colony completely moved into the chamber

Table 2. Mean (±SEM) percent survival of *C. formosanus* workers in each colony pair and in controls in tests using 3 interconnected sand-filled test chambers after 14-d exposure

Colony pair Colony 1/Colony 2	Agonism observed in test[a]		Mean percent survival of workers[b]	
	Petri dish 24 h	Test chambers 14 d	Colony 1	Colony 2
C1: Control	–	–	–	81.1 ± 5.8a
C5: Control	–	–	–	83.1 ± 3.4a
C6: Control	–	–	–	84.5 ± 3.5a
C11: Control	–	–	–	88.1 ± 1.5a
C12: Control	–	–	–	89.6 ± 1.8a
C1/C6	no	no	84.8 ± 2.7	85.2 ± 0.5a
C11/C12	no	no	87.9 ± 1.3	86.4 ± 3.6a
C5/C6	no	yes	71.6 ± 7.3	71.7 ± 6.4ab
C1/C11	yes	yes	69.3 ± 8.0	60.0 ± 12.1ab
C1/C5	no	yes	70.4 ± 12.2	61.4 ± 10.8abc
C6/C11	yes	yes	47.2 ± 7.3	44.7 ± 14.9bcd
C5/C11	yes	yes	54.6 ± 3.9	16.9 ± 11.9cde[c]
C5/C12	no	yes	32.7 ± 15.9	46.1 ± 20.4de
C1/C12	no	yes	4.6 ± 4.6	20.7 ± 11.9e

[a] If agonism was observed in at least 1 replicate for a colony pairing, the pairing was scored as yes, and if no agonism was observed in any of the replicates for a colony pairing, the pairing was scored as no.

[b] Means within a column followed by the same letter are not significantly different. Differences in worker survival among colonies in controls were not significantly different.

[c] Means within a row are significantly different ($P \leq 0.05$). Worker survival within each colony pair were compared using a *t*-test for matched pairs.

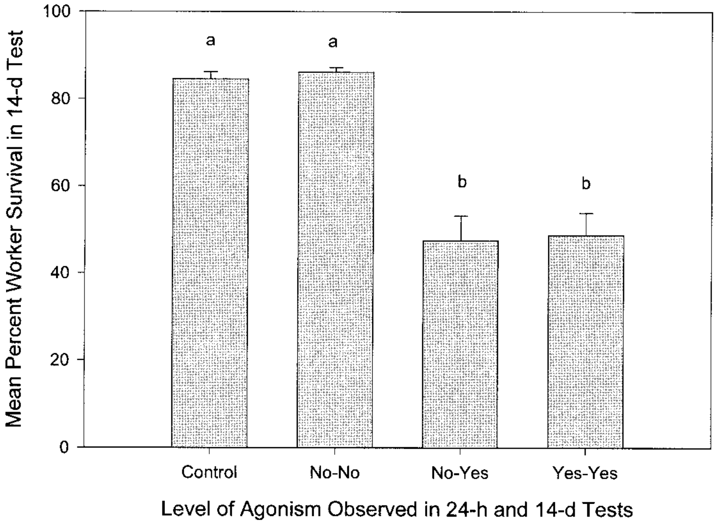

Fig. 1. Mean (±SEM) percent worker survival in the 14-d test for the following groups of colony pairs. Control: Replicates where only one colony was released in test chambers; No-No: colony pairs that did not show agonism in either the 24-h or 14-d tests; No-Yes: colony pairs that did not display agonism in the 24-h petri dish test but did show agonism in the 14-d test; Yes-Yes: colony pairs that displayed agonism in both tests.

occupied by the other colony. For colony pairs that did not show agonism in the 24-h petri dish test but did show agonism in the 14-d test, termites from one colony moved into the other colony's chamber in 34.7% of replicates. In these colony pairs, termites from the two colonies intermingled in most of the replicates. However, fighting between colony members also occurred, and galleries containing mixed groups of dead termites were observed. In some of the replicates where fighting had occurred and mortality was high, groups of surviving termites from the two colonies were still intermingling after 14 d. When the test chambers were dismantled, these remaining termites did not appear to be fighting, although there were dead termites from the two colonies located in another part of the same test chamber. For colony pairs that showed agonism in both tests, termites only occupied the center chamber in 10% of replicates, and there were no replicates where one colony moved into the other colony's chamber. Termites from the two colonies did not intermingle in any of the replicates, and workers were not observed traveling within tunnels constructed by workers of the other colony.

Intercolonial Interactions between Individually Marked Termites. In tests in which individual behavior was observed for 1 h, there was considerable variation in the aggressive behavior of individual workers (Table 3). Even for colony pairs that had showed a high level of agonism in the previous tests, most workers did not display any agonism within the 1-h observation period. The highest percentage of individuals displaying aggressive behavior occurred in replicates, with the colony pair C6:C11. In these replicates, 41.7% of C6 individuals attacked C11 workers (Table 3). There were also differences in the levels of aggression observed among individuals that showed agonistic behavior. For instance, several aggressive individuals persistently attacked alien workers until they were injured, while other aggressive individuals were observed biting alien workers intermittently, without causing noticeable injury. For all colony pairs, there was at least one replicate where no individuals of either colony acted aggressively toward each other. Observations of individual behavior also determined that, at least for these particular colony pairs, aggressive behavior by members of one colony against members of the other colony usually did not elicit any aggressive reaction from the victims of the attacks. Of the 50 replicates in which aggressive behavior by at least one individual was observed, there was only a single replicate where workers from both colonies engaged in mutual fighting. In this replicate, all members of C1 and C11 engaged in fighting, and virtually all termites were killed or seriously injured within

Table 3. Percent individual *C. formosanus* workers from different colonies observed displaying aggressive behavior towards members of the other colony in 1-h petri dish tests

Colony pair[a] (Colony 1/Colony 2)	Percent aggressive workers for each colony pairing	
	Colony 1	Colony 2
C1/C5	1.6	0.0
C1/C6	0.0	0.0
C1/C11	33.3	10.0
C1/C12	38.3	0.0
C5/C6	0.0	0.0
C5/C11	28.3	0.0
C5/C12	0.0	0.0
C6/C11	41.7	3.3
C11/C12	0.0	0.0

[a] For each colony pair, there were 10 replicates with 6 workers from each colony per replicate.

15 min. In another replicate, there were two C11 workers that attacked C6 workers. Otherwise, C11 workers did not engage in any aggressive behavior, even when they were being repeatedly attacked by workers from the other colony.

Discussion

The occurrence of agonism between particular colony pairs was variable. Of the 13 pairs that showed agonism in the 24-h petri dish tests, only five of those pairs showed agonism in all of the replicates. Of the nine colony pairs included in both the 24-h and the 14-d tests, two pairs did not show agonism in either test, four pairs showed agonism in only the 14-d test, and three pairs showed agonism in both tests.

Variability in aggressive behavior of individuals was observed in 14-d tests. Mutual fighting was observed for some colony pairs. In these pairs, workers from both colonies had high levels of mortality. For colony pairs that did not show agonism in 24-h tests but did show agonism in the 14-d tests, surviving termites from the two colonies were continuing to intermingle at the end of the experiment, even in replicates where termites from both colonies had high levels of mortality. However, when C1, C5, and C6 were paired with C11 in the 14-d tests, there were no replicates where termites from the two colonies intermingled. These colony pairs also showed agonism in the 24-h tests, and individuals of C1, C5, and C6 were observed acting aggressively towards C11 workers in 1-h observation tests.

There was variation in the number of individuals that acted aggressively during the 1-h observation period. Most individuals did not act aggressively in these tests. The majority of individuals that acted aggressively were attacking either C11 or C12 individuals. With the exception of a single replicate, these observed attacks did not lead to mutual fighting. In the 1-h observation tests, C11 and C12 individuals did not seem to be able to distinguish colony members from alien workers, even in replicates where they were being attacked, as indicated by their lack of an aggressive response.

Polizzi and Forschler (1998) also found individual variation in agonistic behavior. In a study of agonism using workers of *R. flavipes* and *R. virginicus*, only 4.5% of arenas with intraspecific colony pairs displayed agonism. When agonistic behavior of individual workers of *R. flavipes* and *R. virginicus* was examined, 89% of previously aggressive workers displayed aggression during a second test, while 88% of previously passive workers were passive during a second test (Polizzi and Forschler 1999). These results suggest that there may be a division of labor among termite workers regarding the display of aggressive behavior.

There are several factors that could have contributed to higher levels of agonism in the 14-d test than in the 24-h test. First, numbers of workers from each colony were much higher in 14-d tests. Therefore, there was a higher probability that aggressive individuals were present in the 14-d test. Second, soldiers were present in the 14-d test, but not the 24-h test. It is not known how the presence of soldiers affected the interactions of colony pairs. In tests examining the aggressive behavior of colonies of *Heterotermes aureus* in foraging arenas, the number of soldiers participating in the interactions was critical to the successful invasion of one colony by another (Binder 1988). When the number of soldiers was equivalent, groups from both colonies had close to 100% mortality. When the number of soldiers was disproportionate, the group with the larger number of soldiers successfully invaded (Binder 1988). In a study examining interspecific interactions between *C. formosanus* and *R. flavipes*, groups of *C. formosanus* with higher soldier proportions were more likely to invade territory occupied by *R. flavipes* (Cornelius and Osbrink 2000).

Third, the differences in the duration of the tests may have affected the prevalence of agonistic behavior. In other research, workers of *Reticulitermes* spp. that were not immediately aggressive towards each other caused high mortality after 24 h (Haverty et al. 1999). In the current study, termites from several of the colony pairs that did not act aggressively within 24 h, engaged in fighting during the 14-d test. Therefore, there may be individuals that do not act aggressively towards each other immediately but display agonistic behavior after a longer period. If kin recognition is based, at least partially, on differences in digestive components or in the composition of intestinal bacteria that are detected in the feces or through trophallaxis, termite workers may not immediately recognize workers from other colonies as being alien. Finally, differences in the conditions of the bioassay may have influenced the prevalence of aggressive behavior (Polizzi and Forschler 1998). In the 14-d bioassay, termites were able to forage more naturally by constructing tunnels in the sand and by feeding on wood, while termites were confined in a small arena in the 24-h bioassays.

The kin recognition system of subterranean termites has yet to be described. It has been suggested that the mechanism of kin recognition involves multiple stimuli, including a chemical component, a behavioral component, and a digestive component (Thorne and Haverty 1991). Differences in the composition of cuticular hydrocarbons on the epicuticle of *C. formosanus* were not correlated with intercolonial agonism (Su and Haverty 1991). In studies of agonistic behavior of *C. formosanus* colonies from Hawaii and Florida, geographic distance was not correlated with agonism (Su and Haverty 1991, Shelton and Grace 1997a). Also, aggressive behavior between *C. formosanus* colonies in Hawaii did not correlate with genetic similarities between colonies (Husseneder and Grace 2001a).

Kin recognition cues appear to be affected by environmental factors. Low temperature conditioning reduced intercolonial agonism, presumably due to the elimination or suppression of kin recognition cues (Shelton and Grace 1997b). Colony pairs of laboratory-reared *C. formosanus* did not display any agonism, although these same colony pairs did display agonism when field collected termites were used (Shelton and Grace 1997a). However, agonistic behavior was observed for pairings of workers of *Reticulitermes* spp. from laboratory cultures maintained for > 18 mo, with workers from different colonies that were recently collected in the field (Getty et al. 2000).

Matsuura (2001) proposed that nestmate recognition is mediated by differences in the composition of intestinal bacteria. The composition of intestinal bacteria of *R. speratus* (Kollar) collected from the field was significantly different among colonies. When the composition of intestinal bacteria was experimentally manipulated using antibiotics, the level of aggression was significantly higher among groups given different antibiotics than among groups given the same antibiotics.

Detailed research on agonistic behavior of *Reticulitermes* spp. indicates that there may be multiple stimuli involved. The pairings of workers of *Reticulitermes* spp. with different cuticular hydrocarbon phenotypes usually resulted in immediate aggression, possibly because they were using differences in cuticular hydrocarbons as a kin recognition cue. However, the pairings of workers from different colonies with the same cuticular hydrocarbon phenotype rarely resulted in immediate aggression but often resulted in high mortality after 24 h (Haverty et al. 1999, Getty et al. 2000). Therefore, although workers were able to differentiate immediately between colony members and alien workers with different hydrocarbon phenotypes, workers seemed to be able to differentiate between colony members and alien workers with the same hydrocarbon phenotypes within a 24-h period, even when there was no immediate aggressive response. These results suggest that workers with the same hydrocarbon phenotype may be using other kin recognition cues, such as a digestive or behavioral component, to distinguish between colony members and alien workers.

This study shows that interactions between colonies of *C. formosanus* from the same geographic area are complex and that these interactions could influence strategies for termite control. When there is no agonistic behavior, these results suggest that two colonies would freely intermingle at foraging sites in the field. Therefore, it is possible that more than one colony could be foraging at a bait station or that a large colony was formed due to the fusion of two nonaggressive colonies. The use of deoxyribonucleic acid fingerprinting may provide answers as to whether or not colony fusion occurs in the field (Husseneder and Grace 2001b).

Some agonistic colony pairs clearly avoided interacting with each other. However, other agonistic colony pairs intermingled, resulting in high levels of mortality. Agonistic colonies could potentially, competitively exclude one another from foraging sites, or they could initially colonize the same foraging site and engage in combat. Fierce combat could drive one or both colonies away from a particular foraging site. If neighboring colonies act aggressively towards one another, bait stations within the foraging range of an aggressive colony will probably only eliminate a single colony at a time.

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